

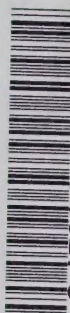
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SURVIVAL OF RAINBOW TROUT, SALMO GAIRDNERI, IN SUBMERGED
ENCLOSURES IN LAKES TREATED WITH NEUTRALIZING AGENTS
NEAR SUDBURY, ONTARIO. 1979.

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TECHNICAL REPORT LTS 79-2

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SURVIVAL OF RAINBOW TROUT, SALMO GAIRDNERI, IN SUBMERGED

ENCLOSURES IN LAKES TREATED WITH NEUTRALIZING AGENTS

NEAR SUDBURY, ONTARIO

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March, 1979

PREFACE

The Ontario Ministry of the Environment in cooperation with the Ontario Ministry of Natural Resources began a comprehensive investigative program in the Sudbury area in 1973 called the Sudbury Environmental Study (S.E.S.). The formal program was initiated in response to earlier studies and reports documenting environmental damage apparently due to smelting operations in Sudbury. Studies include quantification of industrial emissions, atmospheric transformations and transport of those emissions, deposition measurements, determination of chemical and biological lake water quality, developing methods to reclaim acidified lakes and evaluating fisheries in key study lakes. Most of the projects are conducted concurrently by the Air Resources, Water Resources and Laboratory Services Branches of the Ministry of the Environment and by Regional Ministry of the Environment and Ministry of Natural Resources staff.

The Limnology and Toxicity Section, of the Water Resources Branch has been primarily responsible for determining the chemistry and biology of very acidic lakes ($\text{pH} < 4.6$) developing methods of ameliorating acid, heavy metal and low nutrient concentration stresses of lakes and measuring the toxicity of the lake waters to fish.

Lime has been added to four experimental lakes to restore the pH to normal values. Related chemical and biological studies have been carried out and reported previously. This report deals with detailed studies on the survival of fish in the lime treated lakes.

PREFACE

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INTRODUCTION

Acidification of lakes has resulted in the reduction or extinction of fish populations from many lakes near Sudbury, Ontario (Beamish and Harvey 1972, Ontario Ministry of the Environment 1978). In addition to depressed pH, many lakes especially those close to the smelter complex have elevated metal (Cu, Ni, Zn) concentrations (Scheider et al. 1975, Dillon et al. 1977). As the pK's of the metals' hydroxyl complexes are on the alkaline side of neutrality, the major fraction of the metals is in the most toxic, ionic form. If fish communities are to be re-established in these lakes following appropriate "reclamation" procedures, or if we wish to understand, in hindsight, the causes of the population depletions, attention must be given to both of these problems, i.e. to pH and to heavy metals.

Beamish and Harvey (1972), Beamish (1974) and Beamish et al. (1975) have demonstrated that reduction in lake pH alone can lead to extinction of fish in Sudbury area lakes. Scheider et al. (1975, 1976) described neutralization experiments that had raised the pH's of Middle and Lohi Lakes to near neutrality by 1976, i.e. to levels that would not detrimentally affect fish populations (E.I.F.A.C. 1969), decreased heavy metal concentrations, and reduced the proportion of metals existing in the ionic form. As the pH's were no longer critically low, and as available metal concentrations had been reduced, the potential for re-establishing fish communities in these lakes was investigated.

Powell (unpublished studies) stocked Middle Lake with 2500 "young-of-the-year" smallmouth bass (Micropterus dolomieu) in August, 1976 following earlier stocking (June 1976) with 500 Iowa darters (Etheostoma exile) and 200 brook stickleback (Culaea inconstans). At the time, lake pH was 6.2 and total copper, nickel and zinc concentrations were 60, 470 and 35 $\mu\text{g l}^{-1}$, respectively. Despite considerable fishing effort (eight plastic traps for 193 hours, 12 trap nets for 290 hours and 2 gill nets for 51 hours) in June of 1977, Powell failed to capture a single bass.

Similarly, Powell (1977) stocked Lohi Lake in April, 1976 with 1200 yearling brook trout (Salvelinus fontinalis), 1400 Iowa darters and 170 brook stickleback. Lake pH at that time was 6.2 and total copper, nickel and zinc concentrations were 40, 160 and 32 $\mu\text{g l}^{-1}$, respectively.

After failing to recapture any trout in 140 hours of gill netting effort four months after the fish had been planted, he concluded that all of the brook trout had died. He assumed that the primary cause of mortality was copper toxicity.

In order to test the assumption that complete mortality had, in fact, occurred and that elevated copper concentrations in the lakes had resulted in the deaths, experiments providing recovery of all test organisms were necessary. This report describes such experiments performed in 1977 in Middle and Lohi Lakes, two lakes in which fish populations had been eliminated, and Lake Panache, a lake near Sudbury that has not been acidified and is known to support a fishery (Powell, personal communication). These investigations had three interrelated purposes:

- 1) To design and test a submersible enclosure for in-lake experimentation with fish
- 2) To use this enclosure to determine if the water quality of Middle and Lohi Lakes was suitable for the re-establishment of fish populations, and
- 3) To assess causes of fish mortalities, if any did occur.

METHODS

A) Holding facilities, enclosure design and installation and fish transportation

A 4 m diameter, 1 m deep plastic pool (Canadian Tire "Champion") was erected on shore at Lake Panache, filled with metalimnetic water and circulated continually at $\sim 40 \text{ l min}^{-1}$ via a Jacuzzi centrifugal pump. The pool was covered with a $\frac{1}{4}$ " plywood roof to which silvered blanketing had been affixed. The temperature of the holding pool was maintained at $16 \pm 2^\circ\text{C}$ throughout the summer using a Coolflow CF75 cooling unit. The pool bottom was cleared of debris every three weeks.

Yearling rainbow trout (Salmo gairdneri) were obtained from Goossen's Trout Farm Limited, Otterville, Ontario, a hard water hatchery, and held in the holding pool for a minimum of ten days prior to transportation to the lakes. During the holding period, fish were fed in the morning and evening with Ewos #4 sinking trout food. No mortalities were recorded in the holding pool.

Enclosures (2 m x 1 m x 1 m) were constructed of 2" x 4" spruce lumber and painted with blue paint (Benjamin Moore #4230 swimming pool paint). John Leckie Inc. "Ace" nylon netting (1/8", 3/16" or 1/4") was stretched around the frame. A hinged door was built into the top of the cage and an open collapsible cylinder of netting, fitted with a drawstring, was fixed to the cage bottom (Fig. 1). Four such cages were anchored with their tops at 8 m in each of Middle and Lohi Lakes in early May 1977 and tied to rafts. One week later two cages were anchored with the cage tops at 13 m in Lake Panache (location and chemical summary of Lake Panache given by Baksi *et al.* 1977). Cage depths were chosen so that at least some of the cage would be within the metalimnion in the summer.

Water temperature never exceeded 16°C in at least the bottom half of the cages at any time during any experiment. Oxygen concentrations at the depth of the cages were favourable for trout survival at all times (10 ± 1 mg l⁻¹ in Middle Lake, 9 ± 2 mg l⁻¹, in Lohi Lake, 11 ± 2 mg l⁻¹ in Lake Panache).

Fish were taken from the holding pool to the lakes in aerated ("hospital grade" air) containers (0.6 m deep, 0.6 m² surface area) which were filled with water from the outflow of the holding pool, lowered in small nylon mesh-walled boxes and planted in the cages through the top door by divers equipped with SCUBA. The time required to remove fish from the pool, transport them to and suspend them in the lake was slightly less than two hours. The lowering rate was adjusted so that at no time were the fish exposed to a temperature change of $>3^{\circ}\text{C hr}^{-1}$. Changes in temperature between holding pool and top of cage are shown in Table 1. At least ten of the fish that were removed from the holding pool for each experiment were returned to the laboratory where they were processed (methods to be discussed) to determine metal residues in tissues at the start of each experiment.

B) Feeding schedules and experimental design

After the cages had been anchored to the rafts, feeding tubes (5 cm i.d. black polyethylene) were installed with their openings approximately 0.5 m inside prepared holes in the cage tops in two of the four cages in both Middle and Lohi Lakes and one of the two cages in Lake Panache. The experiments were thus designed to have equal numbers of "fed" and "non fed" treatments. Food was never provided to fish in Middle or Lohi Lakes (see footnotes 3 and 4 in Table 1) because mortalities were so rapid (to be discussed). Food was given to the fish in the "fed" Lake Panache treatments (Ewos #4 sinking trout food) three times a week.

Table 1

Summary of selected data on experimental design: run number (R), dates of experiments, change in temperature from holding pool to cage (ΔT), stocking density (D), number of fish cage⁻¹ (N) and number of cages used in each experiment (NC).

Lake	R	Dates ¹	ΔT^2 (°C)	D (g wet weight m ⁻³)	N	NC
Middle	1	June 7 (June 10)	-5	220	40	4 ³
	2	July 12 (July 22)	-1	300	28	4 ³
	3	July 26 (Sept 30)	0	420	28	2
	4	Aug. 16 (Sept. 30)	+2	740	140	1
	5	Aug. 30 (Sept. 29)	+2	400	75	1
Lohi	1	June 25 (June 30)	+2	330	40	4 ³
	2	Aug. 16 (Aug. 22)	+3	190	40	4 ³
	3	Aug. 30 (Sept. 19)	+3	170	40	4 ³
Panache	1	June 2 (Aug. 22)	-	100	26	2 ⁴
	2	Aug. 25 (Sept. 28)	+4	150	40	2 ⁴

¹ upper date is first day of experiment, date in parentheses is last date of experiment

² (+) indicates increase, and (-) decrease in temperature

³ although initial design was for 2 "fed" and 2 "nonfed" cages, no food was provided to any fish

⁴ one case was "fed", and one "nonfed"

Fish were placed into cages on five occasions in Middle Lake, on three occasions in Lohi Lake and on two occasions in Lake Panache (Table 1). For the June 2 to August 22 Lake Panache experiment, the trout were taken from the holding pool and planted directly into the cages. To test the stress imposed on the fish by transport, the fish were placed in the transport containers for the August 25 to September 28 experiment, and driven over local roads for two hours prior to being planted in the cages.

Divers visited the cages daily for a week after the onset of each experiment and as often as deemed necessary or was possible, thereafter. Qualitative observations of any changes in breathing and swimming rates, position in the cages, schooling behaviour and maintenance of equilibrium were made. Mortalities were tabulated and dead fish removed from the cages. Geometric mean survival times (GMST) were calculated for each run (Grande 1967). GMST's were very well correlated ($r^* = 0.99$, $p < 0.01$) with median survival times calculated from log probit plots (Sprague 1969).

C) Tissue sampling and data analysis

The dead fish, collected during each experiment and the "control fish" sacrificed prior to each experiment, were rinsed with distilled water on clean enamel trays. An epaxial muscle sample and whole livers and gills were removed. Tissue samples from different fish that had died at the same time were pooled to provide sufficient sample for analysis, and pooled samples were frozen prior to analysis. Subsequently, samples were thawed, dried at 95°C to constant weight (approximately 20 hours), homogenized, digested and analysed for Cu, Ni, and Zn following methods previously described (Ontario Ministry of the Environment 1975).

Analytical precision, based on quintuplicate analyses was $\pm 3\%$ for Cu at $11 \mu\text{g g}^{-1}$ of Cu, $\pm 10\%$ for Ni at $4 \mu\text{g g}^{-1}$ of Ni and $\pm 3\%$ for Zn at $150 \mu\text{g g}^{-1}$ of Zn. Accuracy was assessed to be $107 \pm 6\%$ for Cu, $105 \pm 6\%$ for Ni and $98 \pm 7\%$ for Zn, by measuring recoveries of known amounts of metal.

It was impossible to calculate accumulation rates of the three metals into

*"r" is the coefficient of determination of a linear regression.

the different tissues by regression analysis, because the amount of data collected was too small. Accumulation rates for each metal into each tissue were thus calculated as

$$A_1 = \frac{100}{n-1} \sum \frac{m_{t_n} - m_{t_{n-1}}}{m_{t_{n-1}}} \cdot \frac{1}{t_n - t_{n-1}}$$

$$\text{and } A_2 = \frac{1}{n-1} \sum \frac{m_{t_n} - m_{t_{n-1}}}{t_n - t_{n-1}}$$

where A_1 is accumulation rate in % day⁻¹

A_2 is accumulation rate in µg g⁻¹ day⁻¹

m is metal concentration in µg g⁻¹ (dry weight)

t is time in days

n is number of days for which tissue analyses were available

D) Lake sampling

Temperature and oxygen profiles and samples for chemical analyses were taken weekly at the deepest site (within 50 m of the cage locations) in Middle and Lohi Lakes and near the cage location in Lake Panache using methods and analytical procedures described by Scheider et al. (1975).

RESULTS AND DISCUSSION

A) Behaviour of Trout in the Cages

Drummond et al. (1973) have documented quantifiable changes in fish behaviour (cough frequency, locomotor activity and feeding behaviour) within 2-24 hrs after exposure to copper concentrations as low as 6-15 µg l⁻¹. The design of our experiments did not allow quantification of any such changes, but a qualitative description was warranted as the same behavioural sequence preceded mortalities in all experiments in Middle and Lohi Lakes.

Throughout the duration of the Lake Panache experiments the trout schooled freely throughout the cages. In Middle and Lohi Lakes, the fish were schooling and moving freely throughout the cages a few minutes after their introduction. Within 24 hours the fish were

Table 2

Average values of selected chemical parameters for lakewater samples collected during each experiment, and GMST of trout in each experiment

Lake	run ¹ number	hardness ²	conductivity ³	H ⁺ μmoles l ⁻¹	Cu μg l ⁻¹	Ni μg l ⁻¹	Zn μg l ⁻¹	chlor a μg l ⁻¹	GMST days
Middle	1	46	185	0.5	67	440	31	1.9	2.3
	2	46	189	0.4	55	370	33	1.1	2.1
	3	46	187	0.3	49	411	25	4.1	3.5
	4	46	186	0.4	46	408	23	4.6	4.2
	5	46	187	0.4	46	400	25	5.4	5.7
Lohi	1	25	77	7.9	46	240	33	1.0	2.2
	2	24	76	4.0	42	220	30	0.9	1.3
	3	24	76	2.5	42	220	30	1.1	3.5
Panache	2	28	75	0.3	6	28	6	-	34

¹see table 1 for dates

²in mg l⁻¹ as CaCO₃

³μmhos cm⁻¹ at 25°C

no longer swimming in schools. Some were at the top of the cage while others remained along the walls but all were swimming in a rapid and seemingly disoriented fashion. Most of the fish were near the cage bottom within two days; some, although alive, were lying on their sides, others occasionally swam upwards but sank rapidly when swimming motions ceased. Prior to their death, the fish remained on their sides despite prodding. At this stage opercular movements were slow, erratic and apparently exaggerated.

This sequence was repeated in all experiments in both Middle and Lohi Lakes and suggests that mortality was caused by some stress, although not necessarily by heavy metal toxicity.

B) Mortalities

Differences in rates of mortality between fed and non-fed treatments in Lake Panache were small. However, differences were apparent between the June and August experiments. In the transported group, there was 10% mortality twenty days after the fish had been planted in the cages. In the non-transported experiment, none of the fish had died fifty days after they were placed in the cages (Fig. 2) although they had lost weight (Appendix 1). Data from the non-transported experiment were excluded from subsequent analyses as these differences in mortality rates could have been the result of transportation.

The Lake Panache data suggest that without stresses related to moving the fish, the cages as designed and installed could support fish for fifty days without mortality.

Mortalities occurred much more rapidly in Middle and Lohi Lakes (Fig. 3) than they did in Lake Panache. GMST's ranged from 2.1 to 5.7 days in the five Middle Lake experiments and from 1.3 to 3.5 days in the Lohi Lake experiments (Table 2). As divers observed the fish only once per day, calculated GMST's must be considered as overestimates of actual GMST's.

GMST's were not correlated ($r = 0.28$, $p > 0.4$) with hydrogen ion concentration (Table 2), with hardness of the lake water (Table 2, $r = 0.24$, $p > 0.5$), with the temperature change between the holding pool and the lake at cage depth (Table 1, $r = 0.5$, $p > 0.1$), with stocking density (Table 1, $r = 0.29$, $p > 0.4$) or with initial fish weight (Appendix 1, $r = 0.24$, $p > 0.5$). Although differences among the three lakes in any of these parameters could be expected to modify dose-response

Table 3

Concentrations ($\mu\text{g g}^{-1}$ dry weight) of copper, nickel and zinc in different fish tissues at the start of each experiment (M_0) and at the GMST (M_{GMST})

M_0 M^2_{GMST}

Lake	Run number	Gill			Liver			Muscle			Gill			Liver			Muscle		
		Cu	Ni	Zn	Cu	Ni	Zn	Cu	Ni	Zn	Cu	Ni	Zn	Cu	Ni	Zn	Cu	Ni	Zn
Middle	1	1	1	16	25	1	16	1	1	4	2	2	18	34	1	19	1	1	4
	2	3	2	95	100	1	81	1	1	18	7	5	90	130	2	89	1	1	18
	3	7	1	88	87	<5	82	2	1	19	8	5	100	190	2	110	1	2	24
	4	ND ¹	ND	ND	ND	ND	ND	ND	ND	ND	7	3	125	240	3	140	1	1	30
	5	7	1	150	300	<4	150	2	1	27	8	6	170	440	5	160	2	1	26
Lohi	1	11	12	100	100	1	97	2	1	21	8	13	98	120	2	100	2	1	21
	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3	7	1	150	330	<4	150	2	1	25	18	6	150	380	4	165	2	1	25
Panache	2	12	2	140	110	9	78	2	1	30	14	5	320	550	10	140	2	1	36

¹ND indicates data was not available

² M_{GMST} calculated by interpolation from accumulation rates (Table 5) and concentration of metals in tissue immediately before and after GMST

Table 4

Summary of significance tests of coefficients of determination (r) of linear regression of GMST's against concentration of metals in lake water (M_L), concentration of metals in tissues at the start of each experiment (M_O), concentration of metals at the GMST (M_{GMST}), and accumulation rate (A in $\% \text{ day}^{-1}$)

Metal	Variable x	Variable y			
		muscle	liver	gill	lake water
Cu	M_L	NA	NA	NA	***
	M_O	-	-	-	NA
	M_{GMST}	-	**	-	NA
	A	-	*	-	NA
Ni	M_L	NA	NA	NA	**
	M_O	-	ID	-	NA
	M_{GMST}	-	-	-	NA
	A	-	-	-	NA
Zn	M	NA	NA	NA	***
	M_O	-	-	-	NA
	M_{GMST}	-	-	***	NA
	A	-	-	-	NA

NA implies test was not applicable

ID implies insufficient data to perform tests. Ni levels in liver at the start of experiments were often below detection limits (Appendix 1)

- $p > 0.1$

* $p < 0.1$

** $p < 0.05$

*** $p < 0.01$

relationships of toxicant mixtures (Harrison 1975, Hodson et al. 1978, Pagenkopf et al. 1974), the regression analyses suggest that such differences (Tables 1 and 2) were not of themselves directly responsible for the differences in GMST between Panache and Lohi and Middle Lakes.

Lett et al. (1976) have shown that rainbow trout can adapt to stresses imposed by sublethal concentrations of heavy metals such as those measured in Lake Panache (Table 2). As the holding period in Lake Panache water prior to each experiment varied from ten days to several months, it is possible that different degrees of adaptation to heavy metal stress could have been developed. Mortality rates in Middle and Lohi Lakes could have been influenced by the degree of adaptation. Brungs et al. (1973) have shown that fish accumulate metals during exposures to sublethal metal concentrations. Thus, initial tissue burdens of heavy metals may reflect differing pre-run exposures. As GMST was not correlated with initial metal concentration in any of the three tissues tested (Table 4) it may be assumed that any acclimation to heavy metals that may have occurred during the holding period at Lake Panache did not influence mortality rates in Middle and Lohi Lakes.

GMST's were inversely correlated with concentrations of copper, nickel and zinc in lakes (Fig. 4, Table 4), suggesting that the lower GMST's in Middle and Lohi Lakes were related to heavy metal toxicity. The heavy metal concentrations were themselves correlated (Cu and Zn, $r = 0.85$, $p < 0.01$, Cu and Ni, $r = 0.87$, $p < 0.01$).

Zinc, and especially copper and nickel concentrations in Middle and Lohi Lakes (Table 2) are much greater than levels considered normal for Precambrian Shield Lakes in Ontario (Dillon et al. 1977). Levels in Lake Panache are also elevated but to a much lower extent than in the other two lakes (Dillon et al. 1977). Many investigators (Rehwoldt et al. 1971, Pickering 1974, Pickering and Henderson 1966, Brown and Dalton 1970, Fitzsimons and Reinke 1977) have confirmed that the toxicity of these metals to fish decreases in the order $\text{Cu} > \text{Zn} > \text{Ni}$.

Despite their significant correlation with GMST's, nickel concentrations of Middle and Lohi Lakes are too low to have of themselves caused the rapid mortalities in these lakes. Willford (1966) reported a 48 hour LC_{50} nickel concentration of 160 mg l^{-1} for rainbow trout tested in water of hardness similar to that of Middle Lake

(42 mg l⁻¹ as CaCO₃). Even in water softer than measured in all three lakes (10 mg l⁻¹) investigators (Ministry of Technology 1966) reported a 48 hour LC₅₀ for rainbow trout of 18 mg l⁻¹.

Similarly, zinc concentrations in Middle and Lohi Lakes are not sufficiently high to have been responsible for the rapid mortality rates observed in these lakes (Beamish 1976, Nehring and Goettl 1974, Lloyd 1961, Grande 1967, Sprague and Ramsay 1965).

In contrast, Black et al. (1976) cite several studies (Lloyd and Herbert 1962, Belinski and Jonas 1973) in which two or seven day LC₅₀ concentrations of copper are 40-60 µg l⁻¹ for rainbow trout in circumneutral pH, soft water. The copper concentrations measured in Middle and Lohi Lakes (Table 2) are similar to these reported LC₅₀ concentrations, therefore, the much shorter GMST's in Middle and Lohi Lakes, compared to Lake Panache, are best explained by copper toxicity.

The possibilities of additive or synergistic toxic effects can not, however, be ignored. The toxicities of zinc and copper are apparently additive at low concentrations in soft water (Lloyd 1961, Sprague and Ramsay 1965, Grande 1967), as may be copper-nickel solutions (Anderson and Weber 1976) or mixtures of all three metals (Brown and Dalton 1970). Thus, zinc and nickel may have contributed to mortalities without being primary causes of death.

C) Accumulation of metals in fish tissue

Although Brungs et al. (1973) concluded that the determination of concentrations of copper in fish tissues could not be quantitatively used to elucidate causes of death in brown bullheads exposed to copper, Brungs et al. and other investigators (Teiji et al. 1967, Benoit 1975, Thompson and Paton 1976) have observed that copper does accumulate in some fish tissues at copper concentrations at which toxicity symptoms, including mortalities, are observed. Hence, tissue samples were analysed to see if metals had accumulated during the experiments, and to see if metal concentrations or accumulation rates correlated with GMST's.

Metal concentrations in muscle tissue did not increase from initial levels to the time of the GMST for any experiment (Table 3). Predictably,

accumulation rates were usually zero, or very low (Table 5). Neither parameter correlated with GMST's (Table 4). Benoit (1975) also found that muscle tissue was not a site of copper accumulation. Bluegills that had been exposed to $162 \mu\text{g l}^{-1}$ of copper for twenty-two months did not have increased copper concentrations in muscle tissue, although accumulation did occur in livers and gills.

Rates of uptake of heavy metals into fish tissue can vary greatly in controlled laboratory investigations. Benoit (1975), for example, observed that although average copper residues in livers of bluegills that had been exposed to 40 and $70 \mu\text{g l}^{-1}$ of Cu were 8 times greater than levels measured in controls, the variation in data was great enough that these differences were not significant. It is, in consequence, not surprising that variations in the changes in metal concentrations from initial levels to levels at the GMST (Table 3) and in accumulation rates (Table 5) were quite large. Some trends were, however, evident in the data.

Concentrations of heavy metals in gill tissue were higher at the GMST in comparison with initial levels in seven out of eight experiments for copper, in all experiments for nickel, but in only three out of seven experiments for zinc (Table 3). Accumulation rates into gills were higher in Middle and Lohi Lakes than in Lake Panache in four of six experiments for copper, in five of six experiments for nickel, but in no case for zinc (Table 5).

Copper residues in liver tissue had increased at the time equal to GMST in all experiments. The concentration in livers from fish in Lake Panache ($550 \mu\text{g g}^{-1}$) was in fact the highest concentration observed at the GMST (Table 3). Zinc concentrations also increased in all cases but proportionally less than copper (Table 3). Three of the reported seven nickel concentrations in liver tissue were below analytical detection limits for tissues analysed at the start of each experiment. Examination of the remaining four data sets, however, indicates nickel residues in liver tissue also increased (Table 3). Accumulation rates (Table 5) of copper and zinc into liver tissue were in all experiments, except for copper in run 3 in Lohi Lake, higher in Middle and Lohi Lakes than in Lake Panache. Accumulation

Table 5

Summary of daily rates of accumulation of heavy metals

Lake	run number	Gill			Liver			Muscle											
		Cu %	Cu $\mu\text{g g}^{-1}$	Ni %	Zn $\mu\text{g g}^{-1}$	Cu %	Cu $\mu\text{g g}^{-1}$	Ni %	Zn $\mu\text{g g}^{-1}$	Ni %	Ni $\mu\text{g g}^{-1}$	Zn %	Zn $\mu\text{g g}^{-1}$						
Middle	1	13	0.2	86	0.1	-2	-0.1	17	0	ND	ND	18	0.6	7	0.03	0	0	-5	-0.2
	2	130	1.4	160	4	1	1	18	20	ND	ND	6	5	0	0	0	0	0	0
	3	0	-0.03	69	0.8	2	2	21	35	30	0.9	8	10	0	0	0	0	-0.06	-0.14
	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	5	13	1	47	0.3	-2	-3	5	9	17	0.9	9	13	0	0	0	0	8	0.2
Lohi	1	-2	-0.4	1	-0.1	-2	-1.8	13	12	ND	ND	4	4	3	-0.05	0	0	3	0.7
	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3	36	4	68	0.9	-3	-3	2	11	0	0	7	10	12	0.3	20	0.2	2	0.4
Panache	2	5	0.06	5	0.1	4	5	2	2	0	0	1	0.9	-0.04	-0.01	0	0	0.6	0.2

ND indicates rate was not calculated

(-) indicates negative accumulation rate

rates for nickel could be calculated for only three of the Middle and Lohi experiments. In two of these, rates were higher than in Lake Panache (Table 5).

Except for the accumulation rates of nickel into gill tissue and copper into liver tissue (Fig. 5), accumulation rates were not correlated with metal concentrations in the lakes. Variations in calculated accumulation rates were sufficiently large, however, that the regressions were not significant (Fig. 5).

D) Relationships between uptake of metals and mortality

There are several reasons why one might not expect the accumulation rates or concentrations in tissues of specific metals to be significantly related to GMST. As have been discussed, the lakes differ in total metal concentrations and in concentrations of other parameters (Table 2) known to influence metal toxicity. The contribution to GMST of additive or synergistic toxic effects would not be reflected in accumulation parameters for individual metals. The relationships between metal accumulation rates and metal concentrations in the lakes were weak, for copper and nickel into liver and gill, respectively, or nonexistent for other combinations. Finally, in cases of acute toxicity, heavy metals need not accumulate in body tissues for mortalities to occur (Skidmore 1970, Burton et al. 1971).

The concentration of copper and nickel in gill tissue at the GMST's was unrelated to GMST (Table 4). Concentrations of zinc in gill tissue at the GMST ($r = 0.89$) and copper in liver tissue at the GMST ($r = 0.72$) were both significantly correlated with GMST (Table 4) but the relationships were not inverse, as might have been expected (Fig. 6). As run number was also correlated with tissue concentration (Fig. 6), these data indicate simply that longer exposures to sublethal metal concentrations in the holding pool at Lake Panache had resulted in greater accumulation of metals in the tissues. As suggested by Brungs et al. (1973) mortality is obviously not directly related to tissue burdens of metals. It could, therefore, be the rate of uptake of metals, not their final concentrations that produced the low GMST's in Middle and Lohi Lakes even though the mortalities in these lakes appeared to be acute, not chronic.

Because copper was the metal most likely to have been responsible

for the rapid mortalities in Middle and Lohi Lakes, and because copper accumulated in liver tissue (Fig. 5) the relationship of accumulation rates of copper into liver tissue with GMST was examined (Fig. 7). The coefficient of determination of this regression ($r = 0.70$) accounted for more of the variation in GMST than any other regression between accumulation rate and GMST, but it was significant at only the 90% level (Table 4). As the GMST's were themselves estimates, and as the variation in uptake rates between experiments in the same lake was large, the weakness of the relationship was anticipated.

In summary, regression analyses of accumulation rates of individual metals with GMST were inadequate for proving cause of death in the experiments. Nevertheless, the conclusion that metal, especially copper, toxicity was responsible for the rapid rates of mortality in Middle and Lohi Lakes appears inescapable.

E) Implications for reclamation of acidified lakes

Reclamation experiments have appeared, in the past, to succeed, at least in the short-term (Scheider et al. 1975, 1976). Addition of neutralizing chemicals has increased lake pH and decreased heavy metal concentrations without affecting lake oxygen or nutrient regimes, and transformed phytoplankton assemblages to ones typical of non-acidic lakes (summarized in Dillon et al. 1977).

The success of the reclamation programme, however, has not been complete. The rapid death of the rainbow trout in the cages in Middle and Lohi Lakes lends support to the conclusion of Powell (1977) that brook trout stocked in Lohi Lake in 1976 had died. The mortality data suggest further that in neither Middle nor Lohi Lake is the water quality currently suitable for the re-establishment of a trout fishery. As the deposition rates of heavy metals are very high in the immediate Sudbury area (Dillon et al. 1977) this condition will not improve. Current loadings of acid are sufficient to maintain acidified lakes in their present state, suggesting that the chemical form of these toxic metals will not change. Loadings of acid are also sufficient to gradually exhaust the buffering capacity and subsequently depress the pH of neutralized lakes without further additions of neutralizing substances. The pH of Lohi Lake in July of 1978, for example, was 4.8 (Yan et al. (unpub. data).

Therefore, the reclamation experiments cannot be regarded as successful since their goal was to restore water quality to the point where historic fisheries could be sustained.

SUMMARY AND CONCLUSIONS

1. The cages, as designed and installed proved useful for short-term in situ experiments designed to measure the survival of rainbow trout in three lakes.
2. Rates of trout mortality were very rapid in Middle and Lohi Lakes. Geometric mean survival times averaged 3.5 days in Middle Lake cages and 2.4 days in Lohi Lake cages. Fish survived for much longer periods of time in Lake Panache, a lake known to support a good fishery. Without stresses related to transportation, the fish survived in the cages in Lake Panache for fifty days before any mortalities were observed.
3. Increased periods of acclimation to the low heavy metal levels of Lake Panache water, did not decrease rates of mortality in Middle or Lohi Lakes.
4. Mortality rates were not positively correlated with concentrations of heavy metals in fish tissues, but rates of accumulation of metals into gill and liver tissue were generally much higher in Middle and Lohi Lakes than in Lake Panache.
5. Despite reductions in heavy metal concentrations that have accompanied experimental neutralization of the lakes, levels of copper and possibly also of zinc and nickel acting in conjunction with the copper, were high enough to have resulted in the observed mortalities.
6. Reclamation experiments appeared to have been successful in the past, but they will not, with current methodologies, restore the water quality of lakes with grossly elevated metal levels to the point where viable fisheries can be re-established.

ACKNOWLEDGEMENTS

It is with pleasure that we acknowledge those persons whose assistance facilitated the completion of this work. Lem Scott and Jim Jones designed, built and installed the cages. We thank Barb Locke for assistance in all aspects of the field work. Gail Beggs, Jim Reinke and Peter Dillon provided many helpful suggestions during the course of the work and together with Gord Craig and Anne Zimmerman reviewed the manuscript. Jim Reinke and Bill Baksi cared for the fish holding facility at Lake Panache. Mike Powell provided some of the fish and lent us the fish transportation container. B. Loescher and D. Russell provided the metal analyses.

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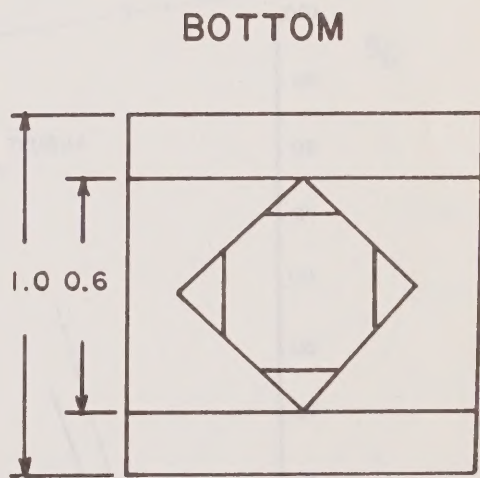
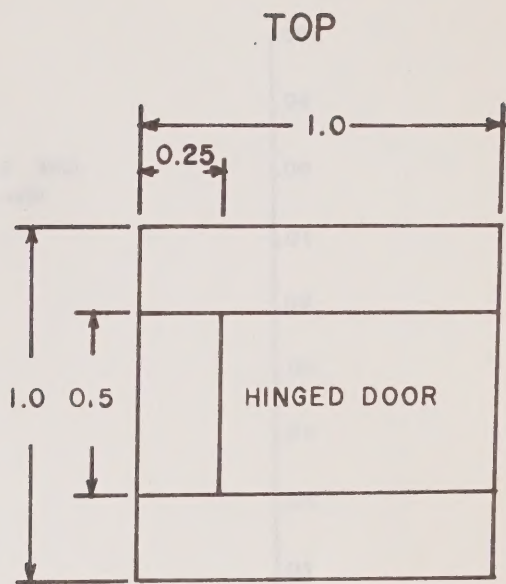
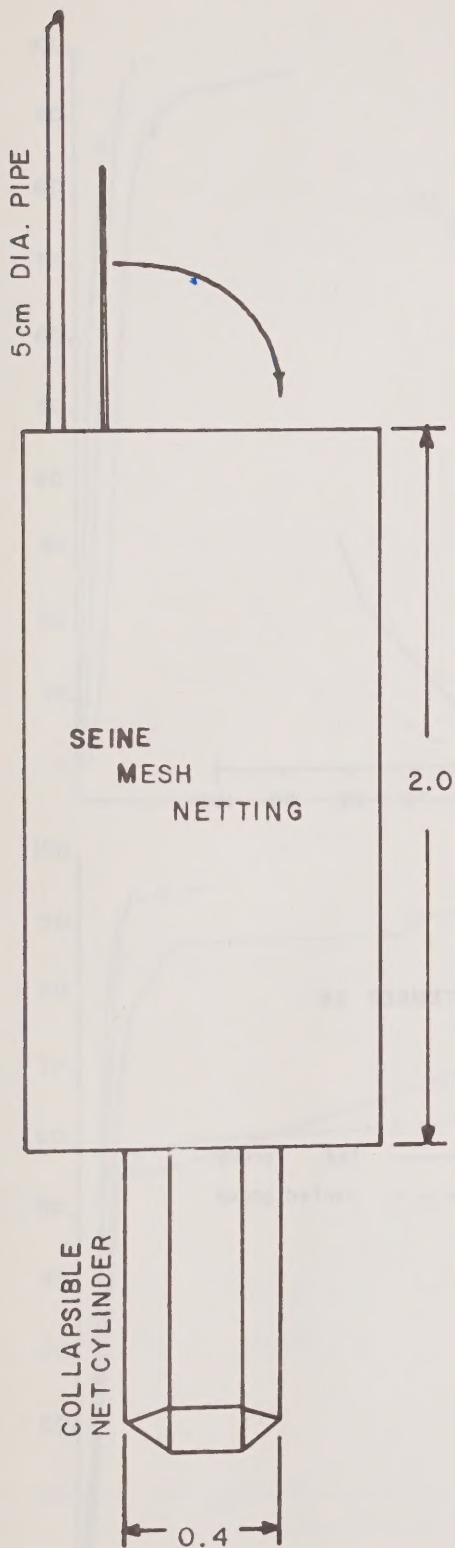
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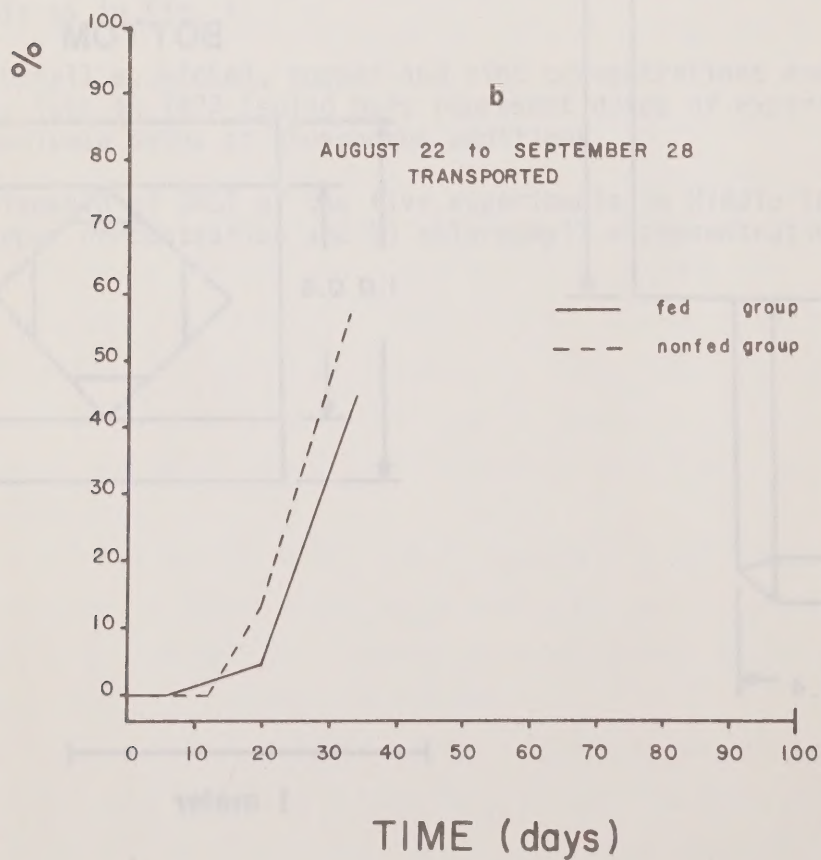
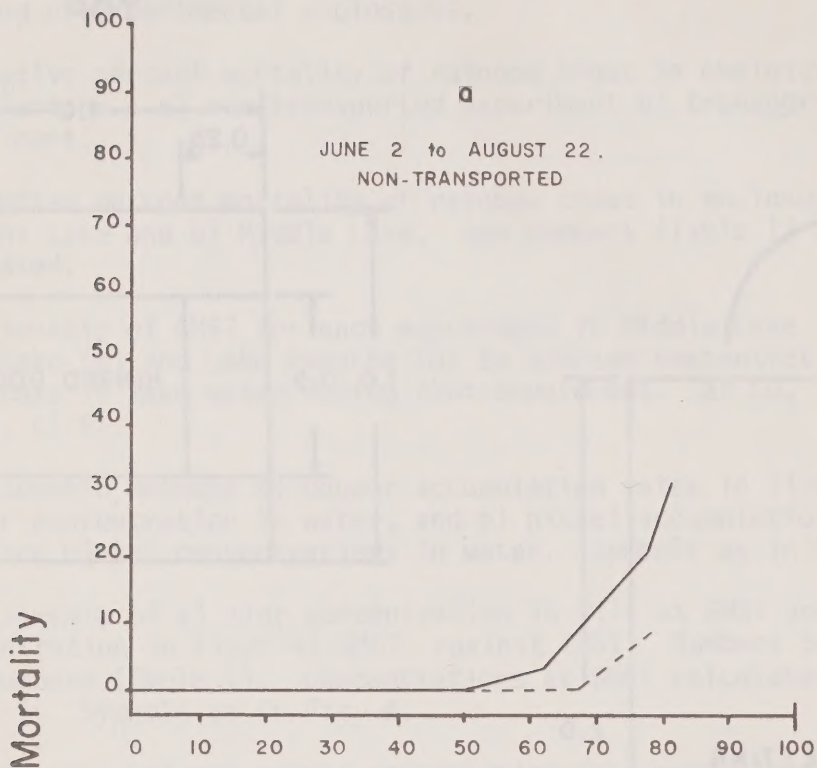
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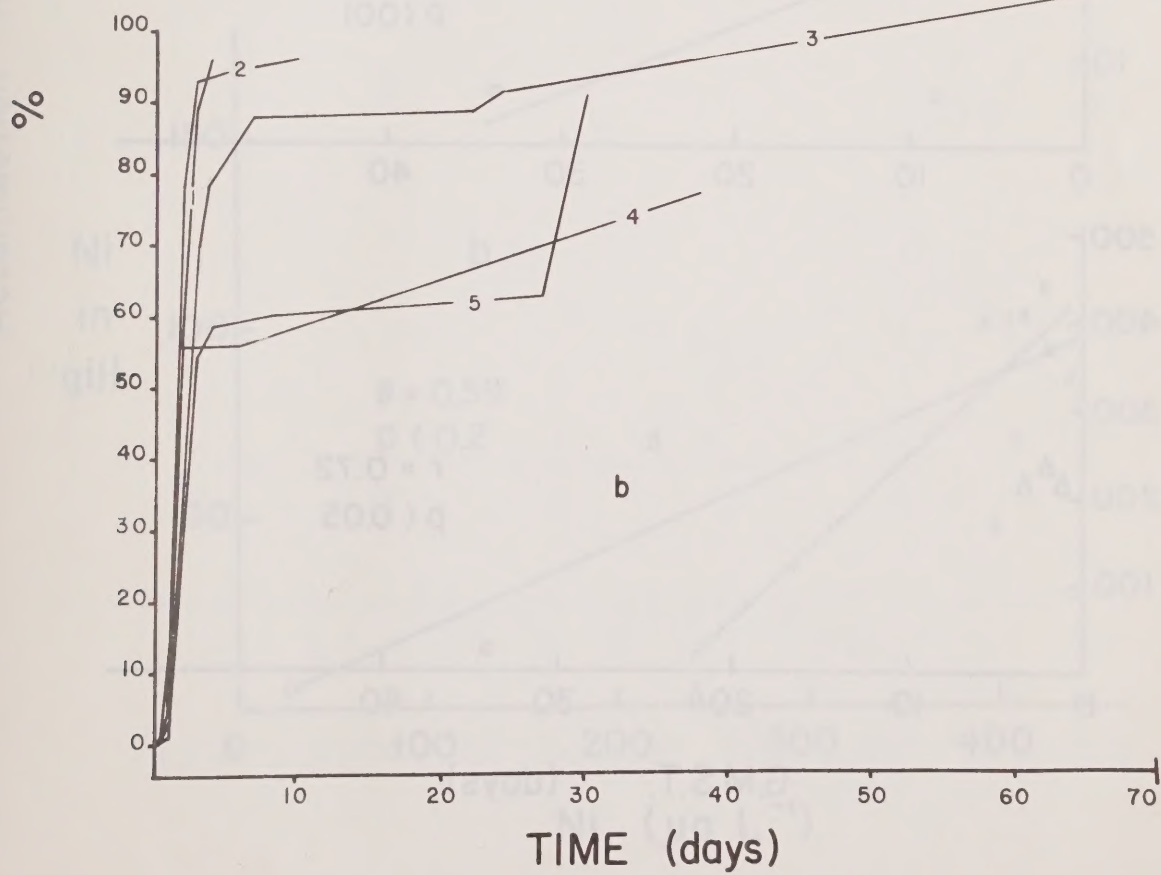
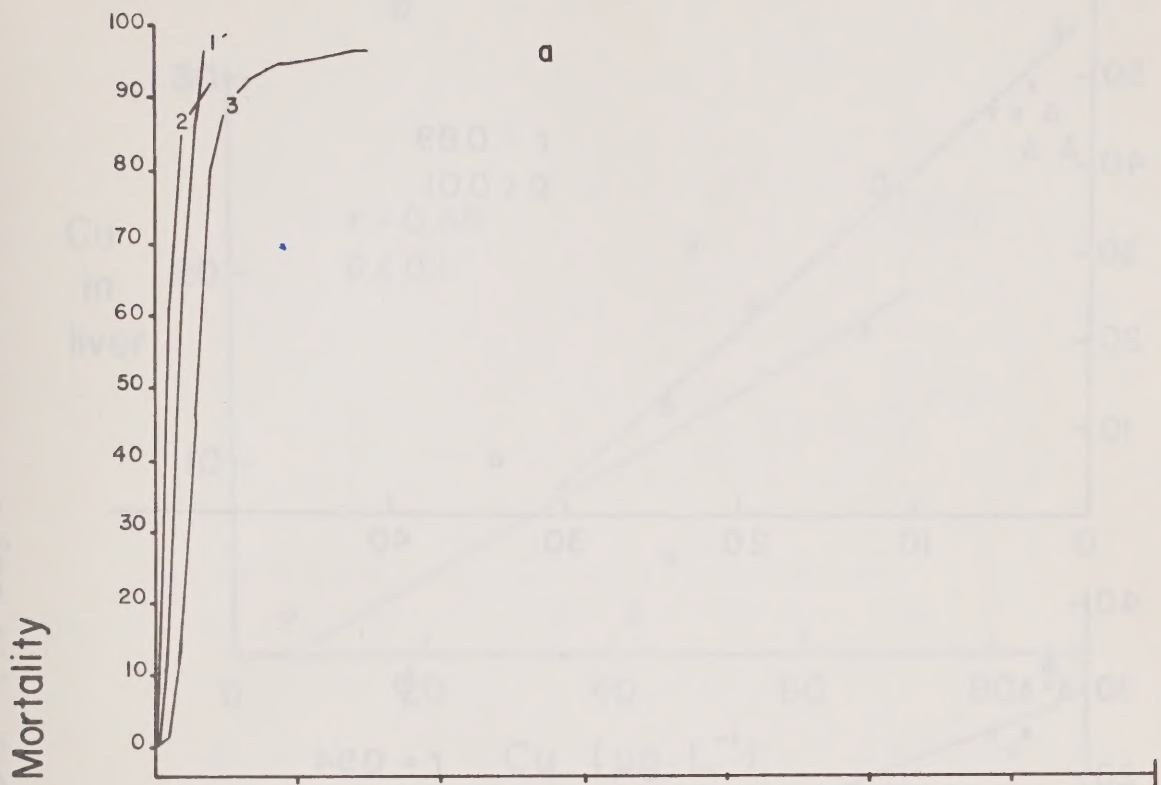
1. Drawing of experimental enclosures.
2. Cumulative percent mortality of rainbow trout in enclosures in Lake Panache. a) non-transported experiment b) transported experiment.
3. Cumulative percent mortality of rainbow trout in enclosures in a) Lohi Lake and b) Middle Lake. Run numbers (Table 1) are indicated.
4. Relationship of GMST for each experiment in Middle Lake (x), Lohi Lake (Δ) and Lake Panache (o) to average concentration of metals in lake water during that experiment. a) Cu, b) Zn, c) Ni.
5. Relationship between a) copper accumulation rates in liver and copper concentration in water, and b) nickel accumulation rates in gill and nickel concentrations in water. Symbols as in Fig. 4.
6. Relationship of a) zinc concentration in gill at GMST and b) copper concentration in liver at GMST, against GMST. Numbers beside points are run numbers (Table 1). Concentrations at GMST calculated as in Table 3. Symbols as in Fig. 4.
7. Relationship between copper accumulation rate into liver and GMST. Symbols as in Fig. 4.
8. Chlorophyll a, nickel, copper and zinc concentrations and pH of Middle lake in 1977 (solid bars represent dates of experiments, "◆" indicate dates of phosphorus additions).
9. Relationship of GMST of the five experiments in Middle Lake to a) copper concentration and b) chlorophyll a concentration of the water.

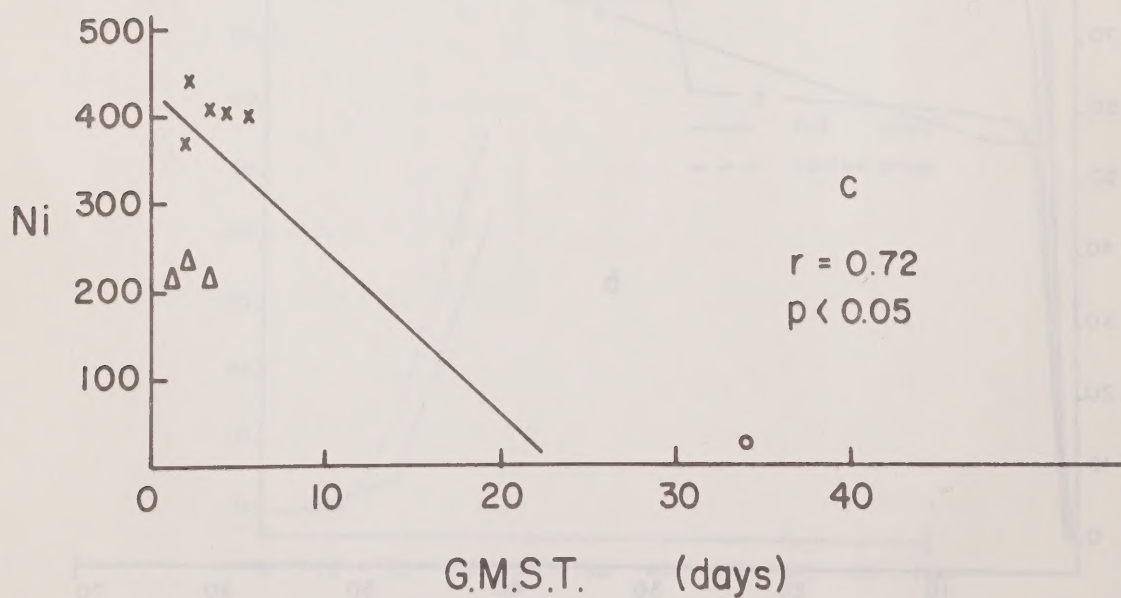
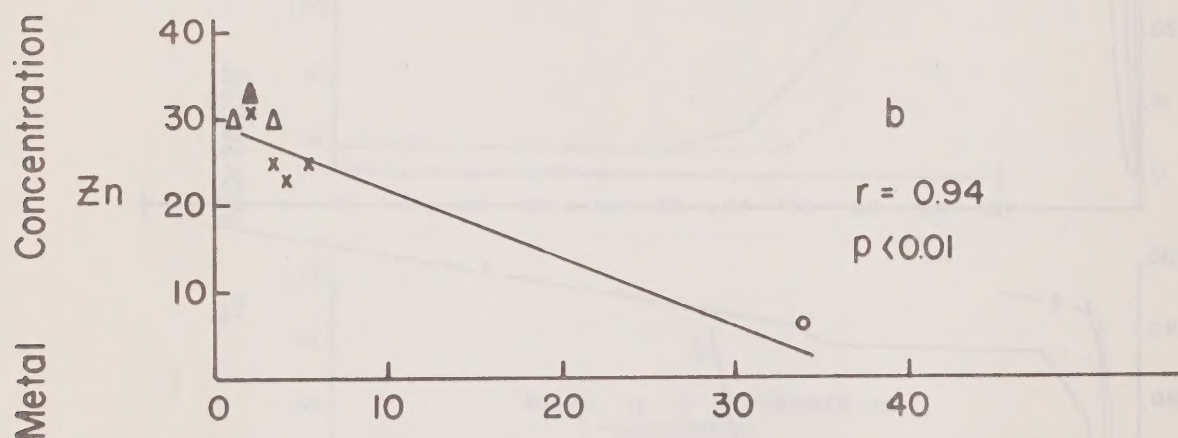
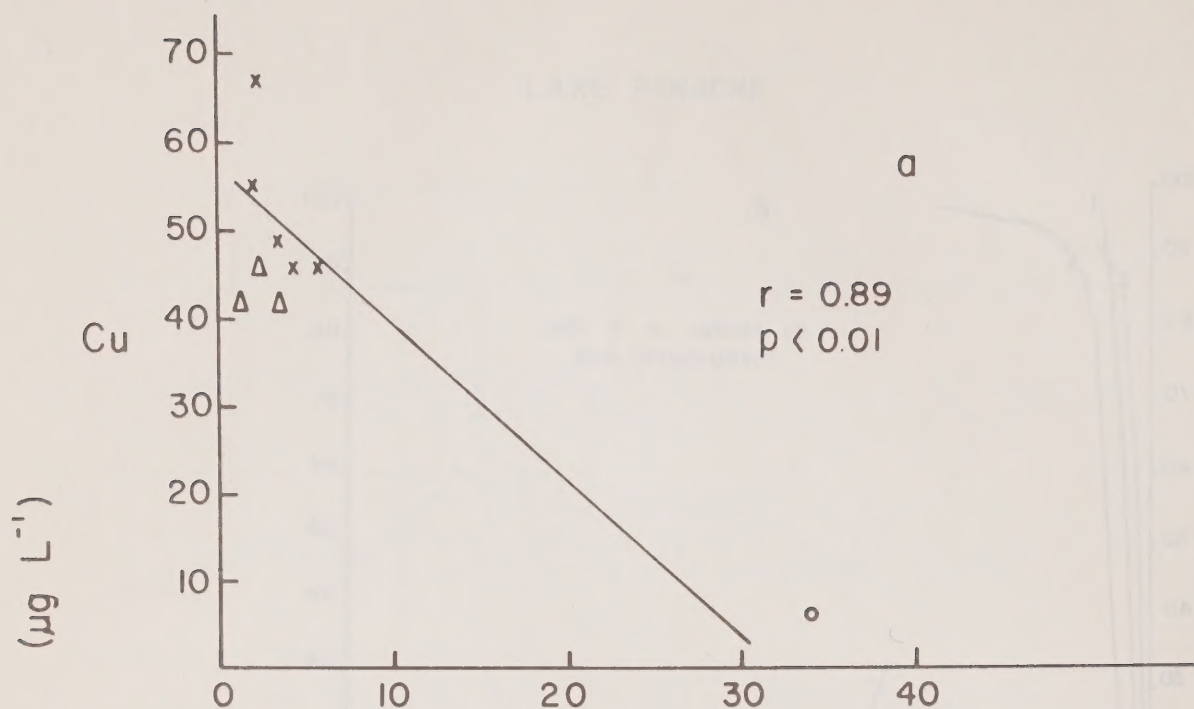


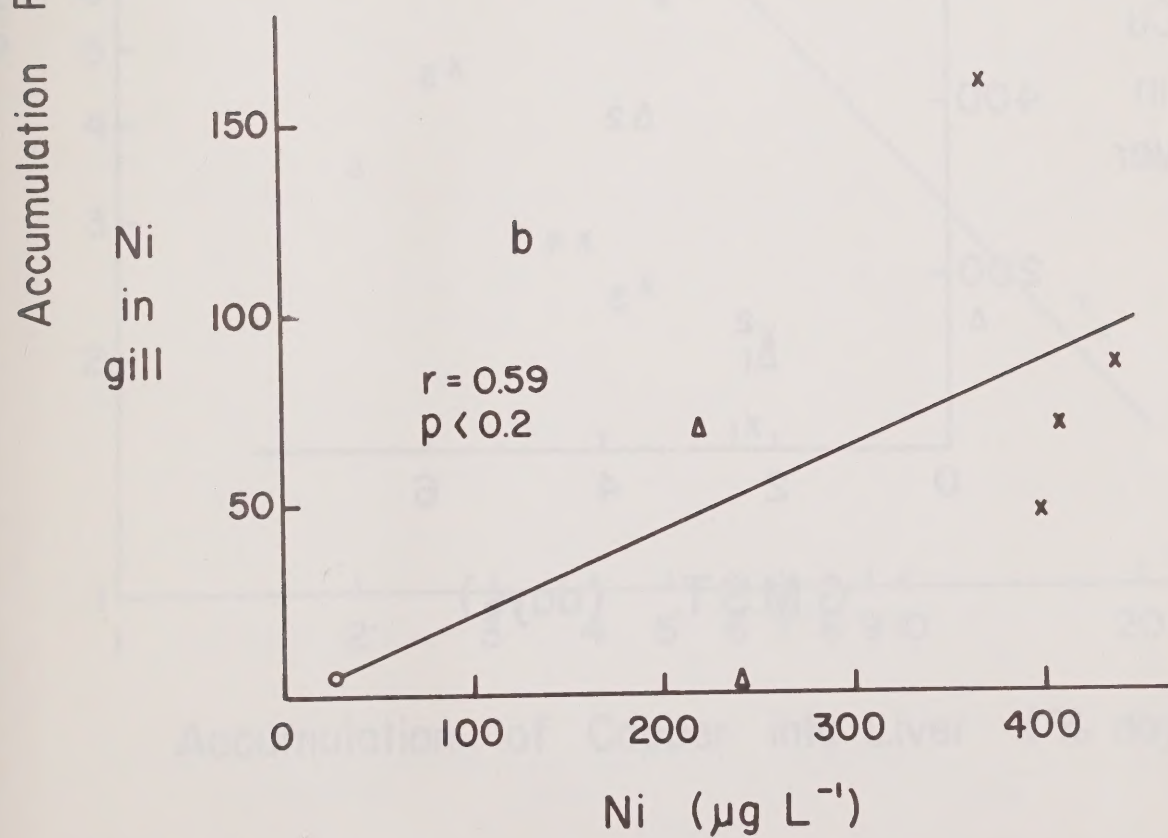
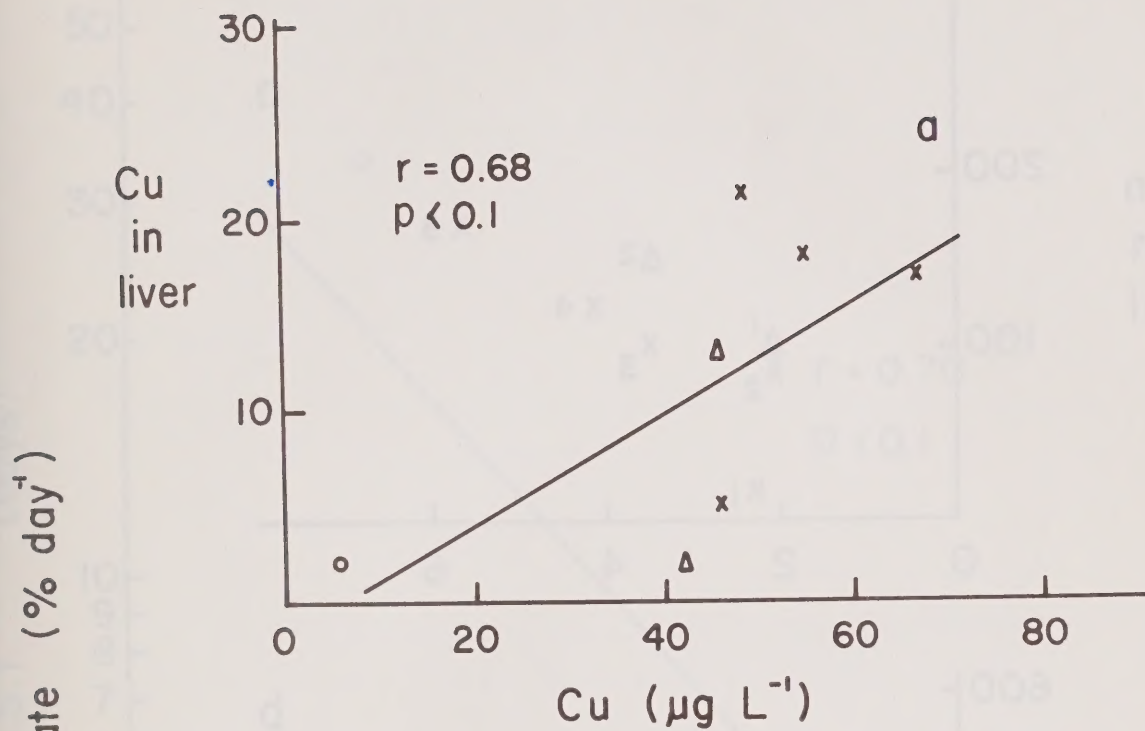
1 meter

LAKE PANACHE

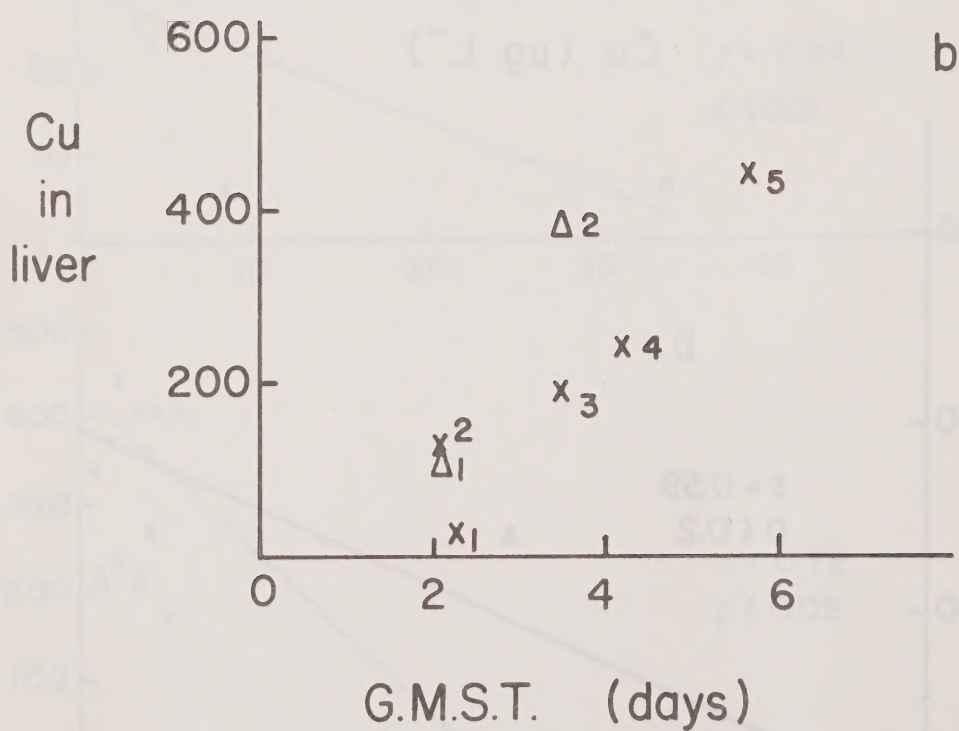
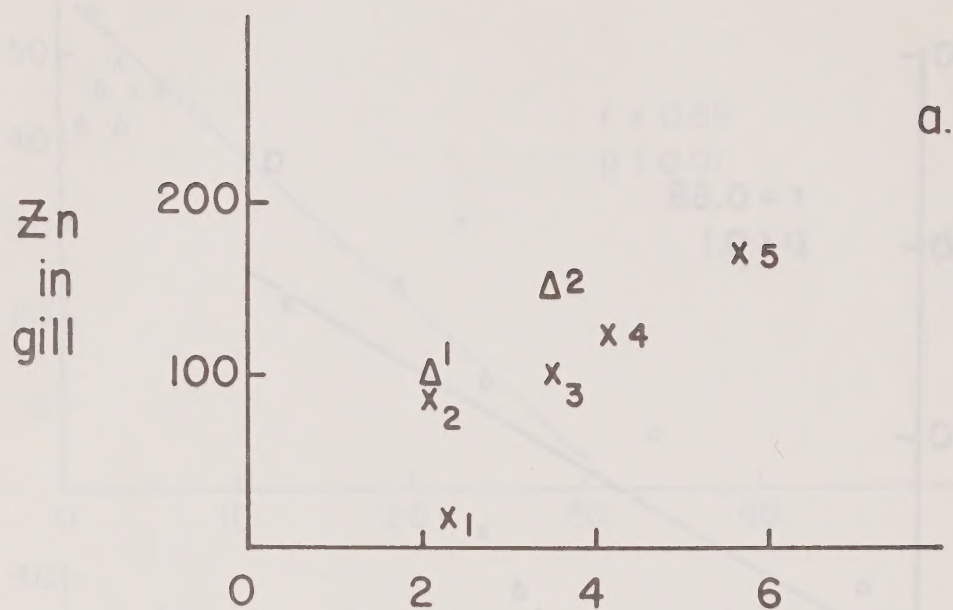




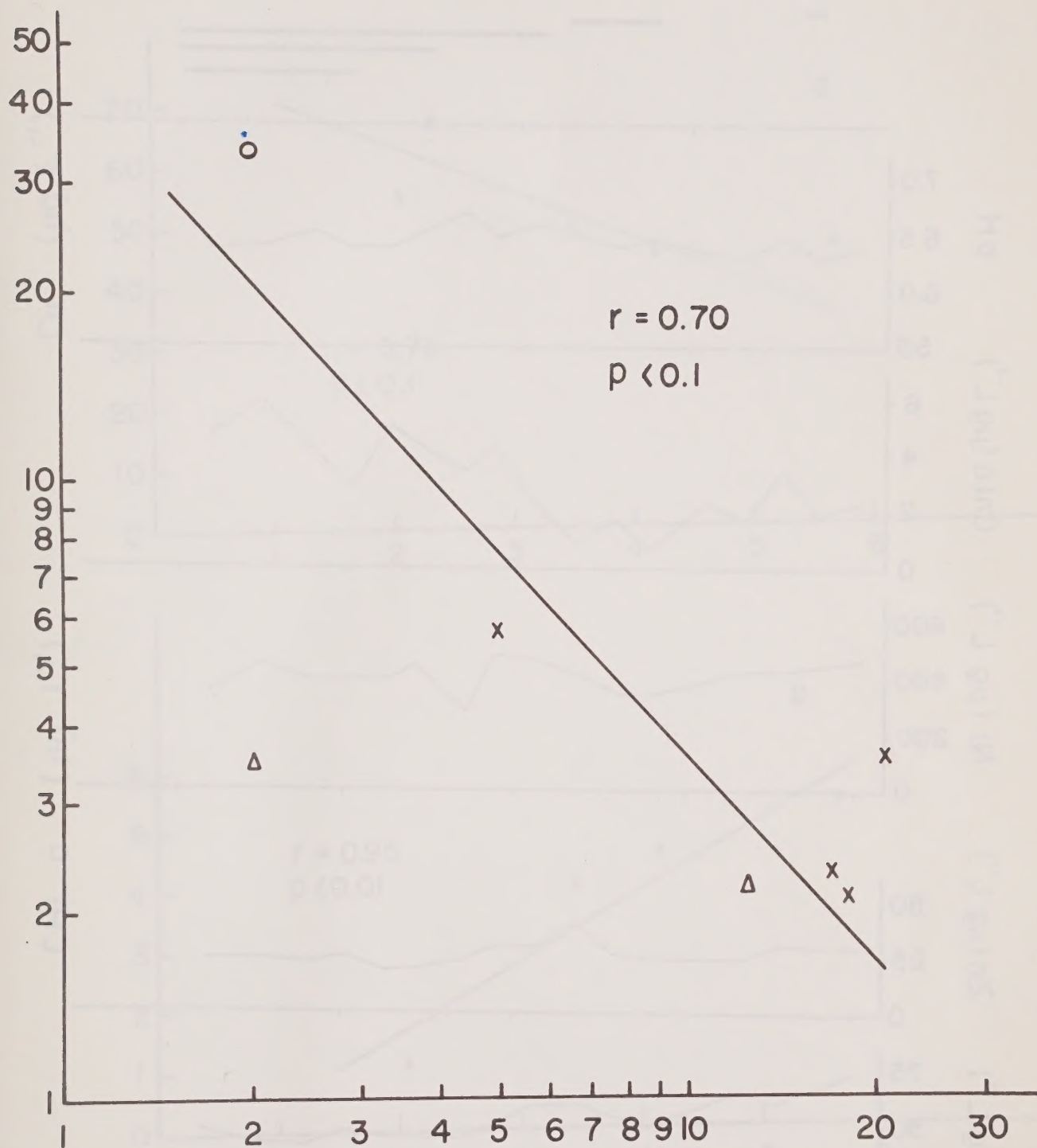




Tissue Metal Concentration at G.M.S.T. ($\mu\text{g g}^{-1}$)

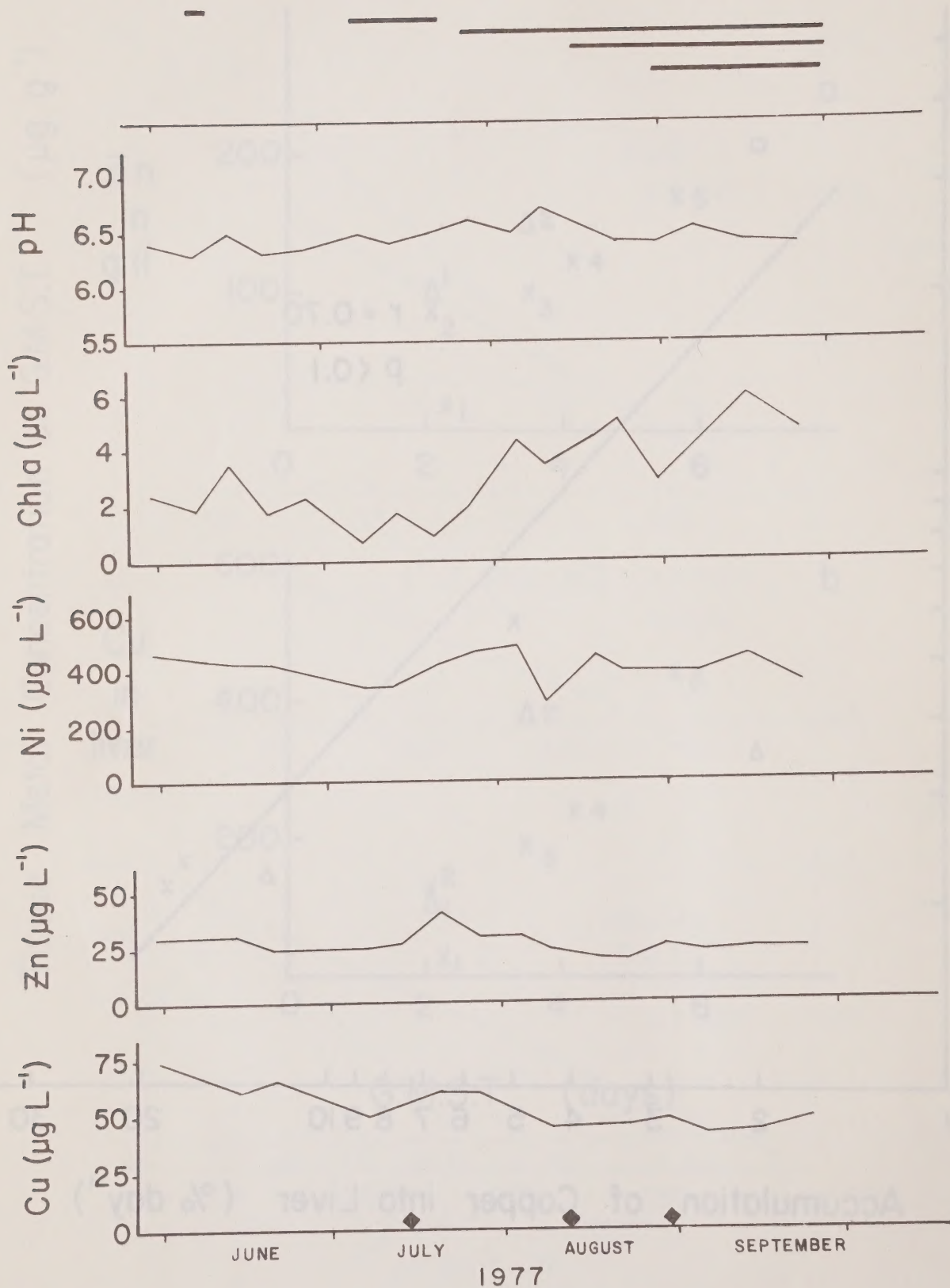


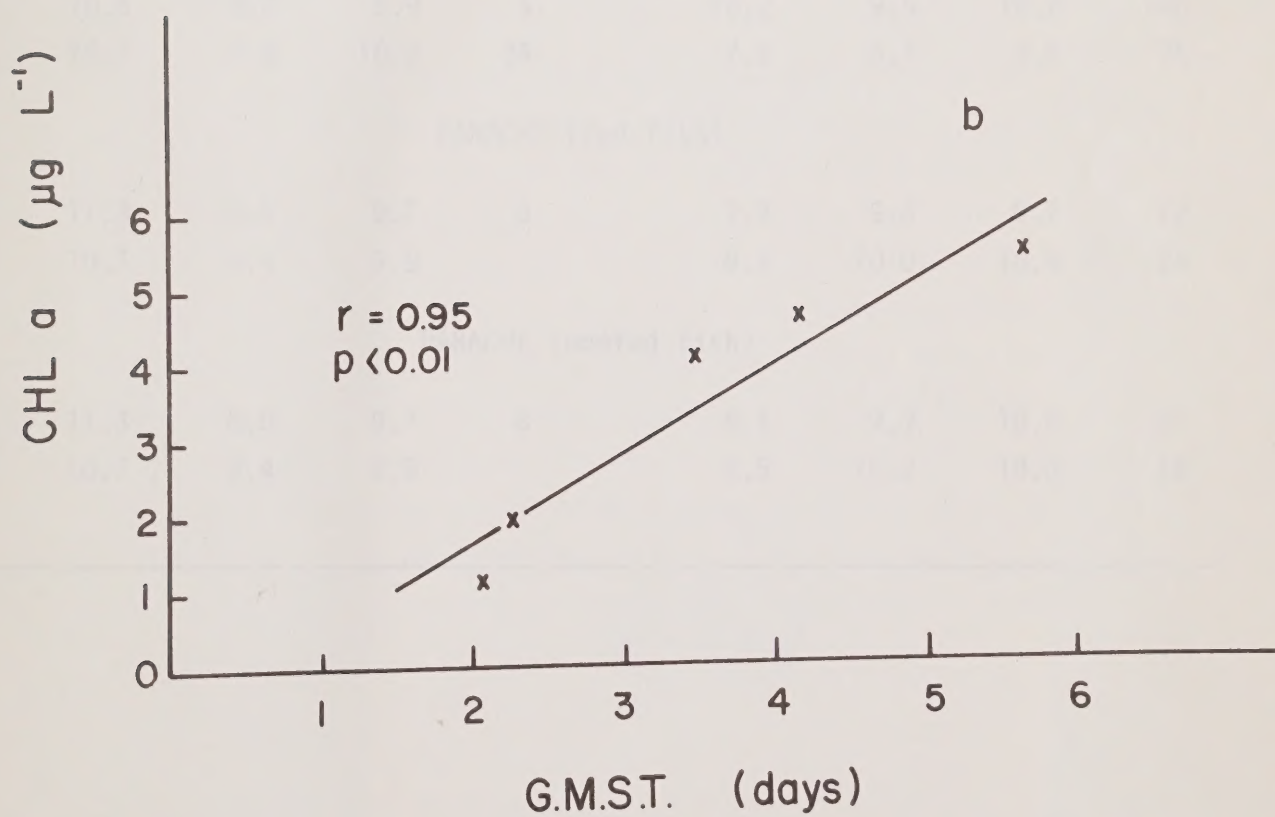
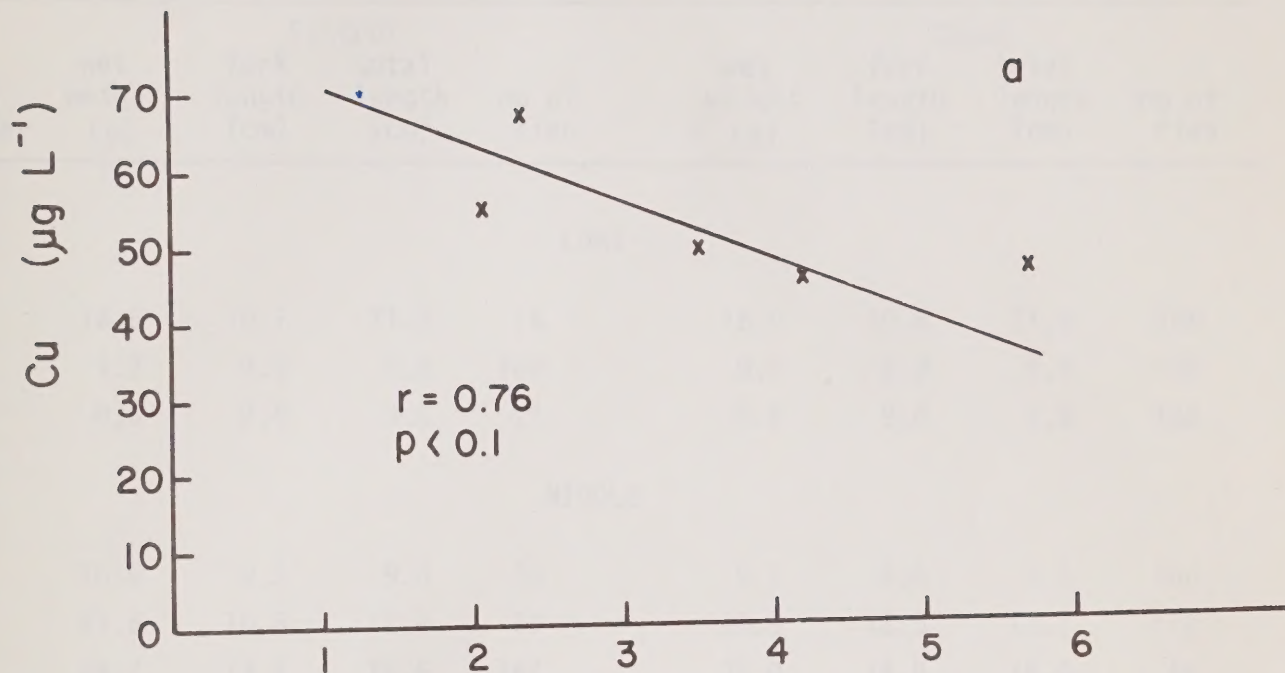
G.M.S.T. (days)



Accumulation of Copper into Liver (% day⁻¹)

MIDDLE LAKE





APPENDIX 1

Wet weights and lengths of control fish (taken from pool at beginning of each experimental run) and caged fish

run number	wet weight (g)	Control		no of fish	wet weight (g)	Caged		no of fish
		fork length (cm)	total length (cm)			fork length (cm)	total length (cm)	
LOHI								
1	16.5	10.7	11.3	19	16.0	10.6	11.2	160
2	9.3	9.0	9.6	104	9.0	8.9	9.5	160
3	8.6	9.0	9.6	21	8.6	9.0	9.6	156
MIDDLE								
1	10.8	9.3	9.8	28	9.1	8.8	9.3	160
2	21.5	10.8	11.4	59	26.9	12.5	13.1	112
3	29.7	13.2	14.6	167	38.0	14.5	15.4	56
4	10.6	9.2	9.9	5	10.2	9.5	10.2	140
5	10.7	9.6	10.2	24	7.2	8.7	9.5	75
PANACHE (fed fish)								
1	11.3	8.8	9.7	8	7.2	9.0	9.7	22
2	10.7	9.4	9.9		8.1	10.0	10.6	24
PANACHE (nonfed fish)								
1	11.3	8.8	9.7	8	8.2	9.9	10.6	21
2	10.7	9.4	9.9		8.8	10.2	10.0	16

Appendix 2

Variation in Mortality Rates in Middle Lake Experiments

GMST's increased steadily from the second to the fifth experimental run in Middle Lake (Table 2). While nickel and zinc concentrations and pH did not vary substantially over the course of the summer, copper levels declined from $\sim 65 \mu\text{g l}^{-1}$ to $\sim 45 \mu\text{g l}^{-1}$ (Fig. 8). In response to fertilization experiments, a continuation of those described by Scheider *et al.* (1976), chlorophyll *a* levels increased over the course of the summer (Fig. 8). Changes in copper and chlorophyll *a* concentrations were significant ($p < 0.01$) in a one-way analysis of variance considering experimental runs as treatments. GMST's increased with decreasing copper concentrations (Fig. 9a, $r = 0.76$, $p < 0.1$) and with increasing chlorophyll *a* concentrations (Fig. 9b, $r = 0.95$, $p < 0.01$). Analysis of covariance suggested that of copper and chlorophyll *a* concentrations, changes in the latter were more important contributors to the increases in GMST observed in the later experiments ($r_{\text{GMST, Cu} \cdot \text{chlor } a} = 0.1$, $r_{\text{GMST, chlor } a \cdot \text{Cu}} = 0.88$).

It is unreasonable to assume that increases in chlorophyll *a* itself would have caused increases in GMST, but increased chlorophyll *a* levels are undoubtedly directly related to other changes that would have followed nutrient additions such as increased rates of volumetric primary production. It is thus possible that a larger proportion of the heavy metals was unavailable during the later experiments in Middle Lake, as rates of secretion of extracellular organic compounds, which would have metal binding capacity (Stiff 1971, Wilson 1972), could have increased in response to increased rates of primary production (Saunders 1972). The greater GMST's in the later runs in Middle Lake may be attributable to an absolute reduction in total copper concentrations and, more importantly, to a reduced availability of the remaining copper.

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Technical Report LTS 79-2

SURVIVAL OF RAINBOW TROUT, SALMO GAIKDNERI, I

ENCLOSURES IN LAKES TREATED WITH HYDROCARBONS

**RESOURCE CENTRE
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